ABSTRACT

FACILITATING PROTEIN FOLDING AND SOLUBILITY BY USE OF PEPTIDE EXTENSIONS

Disclosed herein are novel compositions and methods for enhancing the solubility and promoting the adoption of native folding conformation of a protein or polypeptide expressed by recombinant DNA techniques. One embodiment of the present invention relates to a protein or polypeptide of interest is modified through either carboxyl- or amino-terminal peptide extension, so as to promote folding within host cells. Another embodiment relates to a method for enhancing the *in vitro* renaturation of a protein or polypeptide of interest expressed by recombinant DNA techniques, in circumstances where, following expression, a substantial percentage of the expressed protein or polypeptide of interest is localized within inclusion bodies. Yet another embodiment of the present invention relates to an expression vector comprising a nucleic acid sequence encoding a peptide extension and a multiple cloning site for inserting, in-frame with the peptide extension, a nucleic acid sequence encoding a protein or polypeptide of interest. The peptide extensions of the present invention comprise different amino acid sequences and intrinsic net charges, depending upon the specific species. The total length of the

peptide extensions comprise 61 amino acid residues or less, whereas the net intrinsic charges of the peptide extensions range from about −20 to about −2 and from about −20 to about +2, for peptide extensions fused to carboxyl- and amino-termini, respectively. Primary objectives of the present invention include: (*i*) enhancing the solubility, while concomitantly optimizing the folding, of proteins of interest into their biologically-active conformations in host cells ; (*ii*) characterizing the features of the carboxyl- and amino-terminal peptide extension that are necessary for their protein folding activity within host cells; (*iii*) determining whether these carboxyl- and amino-terminal peptide extensions can promote renaturation of mis-folded proteins *in vitro*; and (*iv*) identifying protein characteristics which determine behavior of the protein as a substrate for the peptide extension-mediated folding described herein.